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Age patterns in leaves used by larvae of the satyrine butterfly *Lethe diana*

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Abstract Larvae of the satyrine butterfly *Lethe dina* feed on blades of bamboo grass *Sasa veitchii* var. *hirsuta* whose blades have a two-to-four-year life span. I studied age class preference by *L. diana*. Both current-year blades and blades from previous years were used as oviposition sites by adult *L. diana* and as food by larvae. The ratio of young to old blades used changed seasonally, and these ratios were consistent with the ratio of young to old blades in available bamboo grass bushes during each season, *i. e.* neither adults nor larvae showed a preference for any age class of blades. Larvae grew better when they were fed young blades compared to old blades, so the fitness of *L. diana* would increase if young blades were selected. It is thought that *L. diana* is not particular about leaf age because it cannot discriminate between young blades and old blades.

Key words Blade age, head-capsule width, larval performance, *Lethe diana*.

Introduction

The chemical and physical properties of leaves vary with phenological age within a plant. In general, young leaves are of higher nutritional quality (Suzuki, 1998; Scheirs *et al*., 2002) and their tissues are less tough (Feeny, 1970; King *et al*., 1998) than those of old leaves. Thus, many phytophagous insects prefer young leaves (Price, 1991; Hayashi *et al*., 1994; de Kogel *et al*., 1997; Cardoza *et al*., 2000; Tokuda *et al*., 2001).

Lethe diana (Butler) is a satyrine butterfly whose larvae feed on the leaf blades of various species of bamboo (Fukuda *et al*., 1984). Females lay a single egg at a time on the under surface of a blade of a bamboo grass, and the larvae grow solitary. A young larva feeds on the blade on the culm on which it hatched and as it grows it moves to other culms. Because a blade of bamboo grass typically has a two-to-four-year life span, a bush of bamboo grass contains blades of various ages. In this study, I investigated the leaf age preferences of *L. diana* adults and larvae, as well as the preference-performance relationship.

Materials and methods

I conducted this study in the Kamigamo Experimental Forest Station (Graduate School of Agriculture, Kyoto University) in central Japan (135°46′E, 35°04′N, 140 m above sea level). The vegetation in this area is mixed second-growth forest and a conifer plantation. The bamboo grass *Sasa veitchii* var. *hirsuta* (Koidzumi), the main food plant of *L. diana* in this area, occurs in scattered clumps on the forest floor (see Ide, 2002).

Lethe diana produces three generations per year in Kyoto. Larvae that have overwintered

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pupate and emerge between May and June, and their offspring, the first generation of the year, appear as eggs and larvae from late May to July. Eggs and larvae of the second generation appear from August to September, and their offspring, the third generation, appear as eggs and larvae beginning in September; this generation overwinters. Because the larval periods of the second and the third generation overlap, I pooled the data of these generations. I investigated eggs and larvae of the first generation (late May to July) and eggs and larvae of the second and third generations (August to November, prior to overwintering).

To assess the proportion of young to old blades in bamboo grass bushes, I sampled culms of *S. veitchii*. From the 20th to the 23rd of every month from April to November 1999, I arbitrarily established four 0.16 m^2 quadrats (three 0.25 m^2 in April) in bamboo grass bushes, and mowed all culms within these quadrats. These culms were classified into young current-year culms and old culms from previous years, and the number of blades on every culm was recorded. Culms with albo-marginated blades were considered old.

From May to November in 1997–1999, tracking censuses were conducted to investigate the age of the blades used by larvae. I searched for eggs and larvae in bamboo grass bushes every few days. I then marked unique dots on the back of each larva using a felt-tipped pen and placed a numbered piece of vinyl tape on the culm in which the egg or larva was found. The larval instar and the age class of the culm (young or old) were recorded. Marked individuals were tracked during the following censuses, and their larval stage and the age class of culms that they occupied were again recorded.

Young and old blades were compared in terms of *L. diana* larval growth. In June 2001, eggs were collected from females captured in Tochû, which is located approximately 10 km northeast of the study area. These females laid eggs on the blades of potted bamboo grass. After hatching, larvae were reared individually at room temperature with fresh cuttings of young or old *S. veitchii* blades. The head-capsule width of each instar and the pupal weights of 48 pupated larvae (33 larvae fed on young blades and 15 on old blades) were measured. The effects of blade age on larval growth were analysed by Mann-Whitney *U* test.

Results

Leaf age use patterns in the field

The sprouting of new culms of *S. veitchii* began in mid-April and was mostly completed by May (Fig. 1a). Because the flush of blades continued until July (Fig. 1b), the ratio of young blades plateaued at about 70% after July (Fig. 1a). First-generation *L. diana* larvae appeared betwen May and July, and larvae of the second and third generations fed from August to October before wintering. The mean ratio of young blades during each three-month period, *i. e.*, 51.2% and 72.0%, were compared to the ratios of young blades of bamboo grass resources available to the first-generation larvae and second- and third-generation larvae, respectively.

The ratios of young blades of bamboo grass on which *L. diana* laid eggs and on which larvae fed on are shown in Table 1. These were similar to the ratio of young bamboo grass blades available to each generation, but only third-instar larvae of the first generation in 1999 used old blades more frequently than young blades. The ratio of young blades that were used differed significantly between generations but did not differ among larval stages (ANOVA, Table 2).

Movements of larvae between bamboo grass culms were observed 33 times in the three years

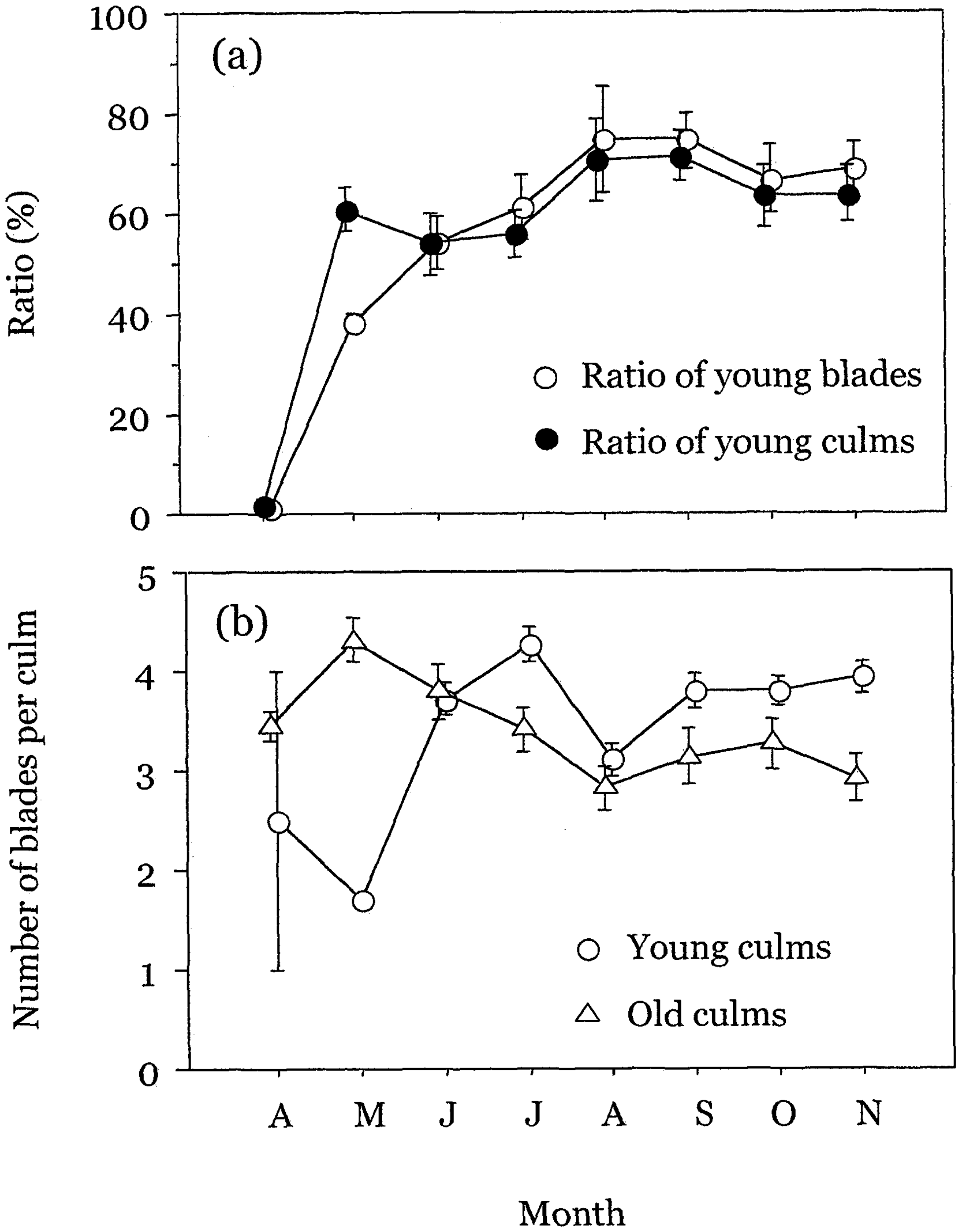

Fig. 1. Seasonal changes in the ratio of young blades and the ratio of young culms (a), and number of blades per culm (b) in *Sasa eitchii* var. *hirsuta* bush in 1999. Means$\pm$SE of were shown.

(Table 3). Movements of third-instar and older larvae were often observed. Larvae of the first generation moved to young culms less frequently than second- and third-generation larvae (Fisher's exact probability test, $P<0.05$). However, in both generations, the proportion of larvae that moved to young culms did not differ from the ratio of young bamboo grass blades (binomial test, NS).

Effect of leaf age on larval growth

Larvae of *L. diana* pupated at the fourth or fifth moult. Fourth-instar larvae pupated when the head-capsule width exceeded 2.9 mm (Fig. 2). The number of moults before pupation differed between blade age classes; 72.7% of individuals that fed on young blades pupated at

Table 1. The ratios of young bamboo grass blades occupied by eggs or each instar larva of *L. diana* (%). Sample sizes are shown in parentheses. The values are compared with the ratios of young blades of bamboo grass resources of each generation (first generation: 51.2%; second and third generation: 72.0%) by binomial test with Bonferroni method ($\alpha = 0.002$).

Year	Generation	Egg	1st instar	2nd instar	3rd instar	4th or 5th instar
1997	1st	53.3 (30)	43.3 (30)	64.7 (17)	71.4 (14)	100.0 (1†)
	2nd & 3rd	85.7 (49)	83.0 (47)	82.9 (35)	80.0 (25)	100.0 (1†)
1998	1st	60.0 (25)	61.5 (26)	61.5 (26)	66.7 (12)	100.0 (2†)
	2nd & 3rd	70.0 (10)	70.0 (10)	76.9 (13)	80.0 (5)	100.0 (1†)
1999	1st	47.4 (19)	45.8 (24)	41.2 (34)	13.9* (36)	52.6 (19)
	2nd & 3rd	80.0 (10)	81.8 (11)	88.9 (9)	57.1 (7)	66.7 (3†)

$^*P < 0.0001$. †When sample sizes are under four, the values are not tested.

Table 2. ANOVA table for the effect of generation and developmental stage on the ratios of young blades where eggs or each instar larvae of *L. diana* occupied.

Factor	d.f.	Mean-square	F	P
Generation	1	3891.31	18.93	<0.001
Developmental stage	3	64.88	0.32	NS
Interaction	3	11.92	0.06	NS
Error	16	205.56		

Table 3. Age classes of the culms that the larvae moved to.

Generation	Age class	Number of movements				
		1st instar	2nd instar	3rd instar	4th or 5th instar	Total
1st	Young	0	2	5	3	10
	Old	1	3	9	2	15
2nd & 3rd	Young	0	4	3	0	7
	Old	0	0	1	0	1

the fourth moult, whereas 33.3% of individuals that fed on old blades pupated at the fourth moult; this difference was significant (Fisher's exact probability test, $P < 0.05$). The head-capsule width of the last-instar larvae did not differ between blade age classes, but that of the fourth-instar larvae did differ; larvae provided with young blades had larger head capsules (Table 4). The pupal weight of individuals fed young blades was also heavier than that of individuals fed old blades (Table 4).

Discussion

Both young and old bamboo grass blades were used for oviposition sites by *L. diana* adults and for food by *L. diana* larvae. The ratio of young blades that were used changed seasonally, from approximately 50% in the first generation to 70–80% in the second and third generations; these ratios were consistent with the ratio of young blades of bamboo grass bushes sampled in each season. The proportion of larvae that moved to young culms also did not differ from the ratio of young blade of bamboo grass in both generations. These results indicate that neither adults nor larvae showed a preference for either age class of blades.

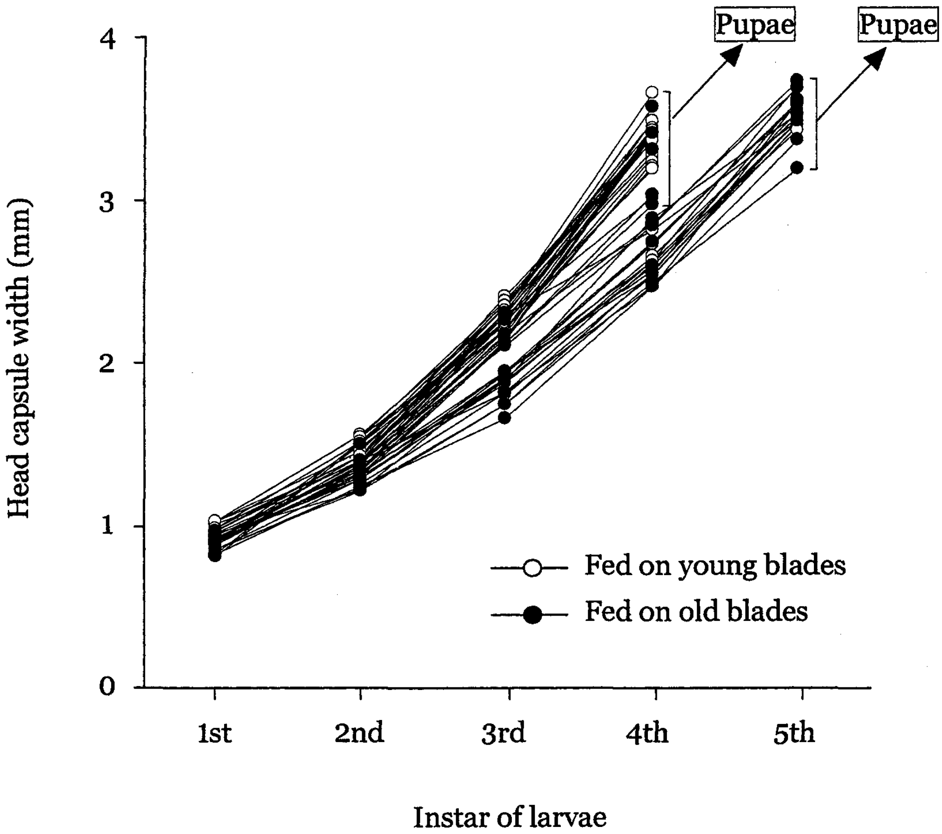

Fig. 2. Growth of head-capsule sizes of *Lethe diana* larvae.

Growth differed between larvae that fed on young blades compared to larvae that fed on old blades. The larvae that fed on young blades had heavier pupal weights, and most required four moults to pupate, whereas many that fed on old blades needed five moults. In the Lepidoptera, larvae with head capsules smaller than a threshold size moult to an additional larval instar, and do not pupate (Nijhout, 1975, 1981). *Lethe diana* larvae that fed on old blades had smaller head capsules at the fourth-instar stage, and smaller individuals moulted to an additional larval instar. Thus, young blades of *S. veitchii* were higher quality hosts for *L. diana* larvae than old blades. This larval performance pattern is known from many plant-insect systems (*e. g.* Hayashi *et al.*, 1994; Steinbauer *et al.*, 1998; Scheirs *et al.*, 2002).

Lethe diana did not select age classes of bamboo grass blades. Because larval performance was better when they fed on young blades, larval fitness would increase if young blades were selected. In almost all investigated cases, the satyrine butterfly *Zophoessa callipteris* (Butler) and the skipper butterfly *Thoressa varia* (Murray), which also feed on *S. veitchii*, used only

Table 4. Growth of *L. diana* larvae provided with young or old blades of *S. veitchii* var. *hirsuta* as food. Means±SE are represented.

	Young (N=33)	Old (N=15)	U	P
Head-capsule width of the last-instar larvae (mm)	3.41±0.02	3.45±0.06	196.5	NS
Head-capsule width of the 4th-instar larvae (mm)	3.17±0.06	2.87±0.10*	127.0	<0.05
Pupal weight (g)	0.335±0.007	0.269±0.014†	59.5	<0.0005

*N=14. †N=13.

young blades (J.-Y. Ide, unpublished data). It is thought that *L. diana* is not particular about leaf age because it is unable to discriminate young blades from old blades. Further research is necessary to clarify the ability of *L. diana* adults and larvae to discriminate leaf-age.

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摘　　要

クロヒカゲ幼虫の食草利用パターン: 葉の新しさについて (井出純哉)

クロヒカゲの産卵場所及び幼虫の摂食場所を調査した．幼虫が餌としていたチュウゴクザサの葉は開いてから 2-4 年に渡って稈についているが，クロヒカゲはその年に出た当年生の葉も，1 年以上たった旧年生の葉も全く区別せずに利用していた．しかし，旧年生の葉を食べた幼虫は当年生の葉を食べたものより成長が悪く，新しい葉を選んで利用した方が有利であると考えられた．従って，クロヒカゲは成虫も幼虫も笹の葉の新しさを識別する能力を欠いており，新しい葉を選べなかったと考えられる．

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